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# Control of growth and development of the feto-placental unit

Victor KM Han\* and Anthony M Carter†

Classical gene targeting has identified many genes important for fetal and placental development. Null mutation of these genes may lead to fetal growth restriction, malformation or embryonic death. Growth restriction of epigenetic basis can predispose to adult-onset diseases. The mechanisms underlying this process, termed 'fetal programming', are beginning to be understood.

## Addresses

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## Abbreviations

<b>E</b>	embryonic day
<b>HERV</b>	human endogenous retrovirus
<b>HIF</b>	hypoxia-inducible factor
<b>IGF</b>	insulin-like growth factor
<b>IGFBP</b>	IGF binding protein
<b>TGF</b>	transforming growth factor
<b>uNK cells</b>	uterine natural killer cells
<b>VEGF</b>	vascular endothelial growth factor
<b>VEGFR</b>	VEGF receptor

## Introduction

Fetal development entails a series of complex events, including differentiation into embryonic and extraembryonic cell lineages and interaction with maternal tissues through a yolk sac and chorioallantoic placenta. Vasculogenesis and angiogenesis to establish a fetal circulation are among the critical early events. Much current research is focused on understanding the genetic control of these complex interactions. With the advent of transgenic and gene-targeting methodologies to determine the biological roles of genes during development, the mouse has become an increasingly important experimental model. Traditional gene-targeting approaches lead to an annulment of gene expression in early gestation and affect the development of both the fetus and placenta. Many of the genes shown in this manner to be essential for fetal survival affect development of the yolk sac and placenta or the vascular system of the conceptus.

Death of the null mutants may be sudden, as in the case of *Mash2* or *Vcam1* mutations, in which the chorioallantoic placenta fails to form. In other cases, death is preceded by growth retardation. It is important to recognize that these effects on growth are often secondary to a restricted supply of nutrients. However, a few genes have been shown to

control cell replication and overall growth directly. Efstratiadis [1] identified 20 growth control genes in mice and only half of these affected birth weight. Seven of the genes are known to control fetal growth code for insulin, insulin-like growth factors I and II (IGF-I, IGF-II), their receptors and the receptor substrates. There have been no additions to this list over the past year and this review describes our current understanding of how these genes affect the development of placental and fetal systems.

The sequelae of poor fetal growth extend well past the postnatal period. Low birth weight has been linked to adult-onset diseases that include hypertension and non-insulin dependent diabetes mellitus [2]. A second focus of this review will be to explore the endocrine responses of the fetus that may lead to an alteration of fetal growth. This process is referred to as 'programming', a term that encompasses genetic and epigenetic events associated with low birth weight.

## Placental development

Placental structure varies greatly across species and comprises a bewildering array of cell types. One of the earliest events in embryonic differentiation is the formation of trophoblast, which gives rise to all the subsequent trophoblast lineages. These include various forms of cytotrophoblast, in which the cells remain discrete, and syncytiotrophoblast, in which cell fusion has occurred. Cells that invade the maternal tissues in man and primates are called extravillous trophoblasts. Vascularization of the placenta from the fetal side requires fusion of the trophoblastic chorion with the allantois and subsequent penetration of the trophoblast by fetal mesenchyme-bearing capillaries.

In the definitive chorioallantoic placenta of the mouse, there are three principal cell layers: the labyrinth, or exchange area; the spongiotrophoblast layer; and the giant cells. The spongiotrophoblasts arise early in development and are thought to give rise to the other cell types (see also Update). The giant cells at the periphery of the placenta have undergone terminal differentiation and are polyploid.

Gene knockout experiments in mice continue to shed light on the factors involved in placental development (Table 1). One of the earliest events, separation of the embryonic and extraembryonic cell lineages, requires the T-box gene *Eomesodermin*. In *Eomes*<sup>-/-</sup> embryos, the trophoblast fails to differentiate into trophoblast [3]. Cytokeratins play a key role in the further differentiation of the trophoblast. In mice lacking both cytokeratin 8 and 19, the placenta lacks spongiotrophoblast and labyrinthine trophoblast [4]. Establishment of the placental labyrinth requires extensive folding of a sheet of trophoblast cells. This occurs in response to contact with the allantois and

**Table 1****Some genes recently found to be essential for normal growth and development of the yolk sac, placenta and embryo in mice.**

Gene	Gene product	Phenotype of yolk sac and placenta*	Phenotype of embryo*	Refs
<i>Eomes</i>	Eomesodermin, a T-box gene product	Trophectoderm fails to develop into trophoblast	Death at blastocyst stage	[3*]
<i>Talin</i>	Cytoskeletal protein	Ectoplacental and exocoelomic cavities fail to form	Failure of cell migration at gastrulation. Death at E8.5–9.5	[54]
<i>Fzd5</i>	Frizzled protein, a receptor for Wnt signaling molecules	Defective yolk sac angiogenesis and placental vasculogenesis	Normal vasculogenesis in embryo. Death at E10.75	[55]
<i>Krt2-8, Krt1-18, Krt1-19</i>	Cytokeratins 8, 18 and 19	Double knockout of 8 and 19: giant cells fail to differentiate into spongiotrophoblast and labyrinthine trophoblast Double knockout of 18 and 19: giant-cell necrosis	Death at E9.5	[4,56]
<i>Foxf1</i>	A forkhead transcription factor	No vasculogenesis in yolk sac or allantois. Chorioallantoic fusion fails and amnion does not expand	Reduced mesodermal proliferation in primitive streak and somite formation retarded. Death at E10	[57]
<i>Epas1</i>	HIF-2 $\alpha$ , a basic helix-loop-helix PAS transcription factor	Vascular defects in yolk sac due to failure of vascular remodeling following vasculogenesis	Vascular defects due to failure of vascular remodeling. Fetal death at E9.5–12.5	[13*]
<i>Tgfb<math>\beta</math>1</i>	TGF $\beta$ type 1 receptor	Impaired yolk-sac vascularization (cause of death) and inhibition of placental angiogenesis	Neural tube defects. Death at around E10.5	[58]
<i>Gcm1</i>	Glial cells missing 1, a transcription factor	Allantois fuses with chorion but labyrinth fails to develop	Death at E9.5–10	[5*,59]
<i>P38<math>\alpha</math></i>	P38 $\alpha$ MAP kinase, a signaling molecule	Reduced spongiotrophoblast, loss of labyrinth, defective yolk-sac angiogenesis	Defective angiogenesis. Reduction of myocardium. Death at E10.5–12.5	[6*,60]
<i>Map3k3</i>	MEKK3, a signal transduction molecule in the p38 $\alpha$ MAP kinase cascade	Disruption of placental angiogenesis and of structural integrity of yolk sac	Death at E11	[61]
<i>cRaf-1</i>	Raf kinase, a component of the ERK cascade	Reduction of labyrinth and spongiotrophoblast. Poor vascularization	Liver small, hypocellular and with numerous apoptotic cells. Anaemia. Death at E11.5–13.5	[62]
<i>Fra1</i>	AP-1 transcription factor	Placental labyrinth largely avascular. Defects in yolk sac	Severe growth retardation. Death at E10–10.5.	[63]
<i>Arnt</i>	Nuclear translocator that dimerizes with HIF-1 $\alpha$	Abnormal development of labyrinth with decreased VEGFR2 and VEGF binding	Embryonic blood vessels develop normally. Death at E10.5.	[12*]
<i>Gab1</i>	Docking protein, binds cMet receptor kinase	Placental labyrinth severely reduced	Impaired migration of myogenic cells. Reduced liver size. Death at E13.5–18.5	[64]
<i>Abca1</i>	ATP-binding cassette transporter	Disrupted placental architecture in <i>Abca1</i> ( $^{-/-}$ )	Severe growth retardation. Death at E14 due to lack of HDL-C and altered steroidogenesis	[65,66]
<i>ApoA1</i>	Apolipoprotein A1	Not described for <i>ApoA1</i> ( $^{-/-}$ )		
<i>Has2</i>	Hyaluron synthase 2	Yolk sac endoderm and mesoderm fail to fuse and blood vessels are lacking	Severe cardiac and vascular abnormalities and growth restriction. Death at E9.5–10	[14]
<i>Gdf1</i>	Growth/differentiation factor 1 (TGF $\beta$ family)	Not described	Defects related to left–right patterning, including visceral situs inversus and cardiac anomalies. Death at E14.5 or later	[16*]
<i>Smad5</i>	Component of TGF $\beta$ family signaling cascade	Not described	Defects related to left–right patterning. Death at E9.5–11.5	[15]
<i>Adm</i>	Adrenomedullin, first described as a vasodilator	Not described	Cardiovascular abnormalities and extreme hydrops fetalis. Death at E13.5–14.5	[17]

\*Phenotype of null mutants ( $^{-/-}$ ). cMET, hepatocyte growth factor receptor; ERK, extracellular-signal-regulated protein kinase; HDL-C, high-density lipoprotein cholesterol; MAP kinase, mitogen-activated protein kinase; MEKK3, MAP/ERK kinase kinase 3.

requires the transcription factor GCM1 (glial cells missing 1). The labyrinth fails to develop in *Gcm1* $^{-/-}$  mice, resulting in death by embryonic day 10 (E10) [5\*]. The signaling pathways involved in placental development involve p38 $\alpha$  mitogen-activated protein (MAP) kinase. In *p38 $\alpha$*  $^{-/-}$  mice, there is significant reduction of spongiotrophoblast and almost complete loss of the labyrinth

layer [6\*]. These mutants are conspicuous examples of embryonic lethality resulting from disruption of placental components essential for survival.

A key role in trophoblast differentiation has been ascribed to genes of retroviral origin. Such genes comprise about 1% of the human genome and, serendipitously, transcription of

the envelope region of human endogenous retrovirus 3 (HERV-3) was found to occur during normal trophoblast differentiation. BeWo cells stably transfected with the HERV-3 *env* gene undergo differentiation that includes inhibition of cell proliferation, through modulation of the cell-cycle regulators cyclin B and p21, and secrete chorionic gonadotrophin [7]. Another member of the HERV family (HERV-W), with the functional properties of a retrovirus envelope, is expressed specifically in the placenta and encodes a protein termed syncytin [8•,9]. The expression of recombinant syncytin in a variety of cell types induces the formation of giant syncytia and an anti-syncytin antiserum inhibits fusion of a human trophoblast cell line [8•]. These results indicate that this protein is important in the differentiation of cytotrophoblasts into syncytiotrophoblast. Why a retroviral genome became incorporated into a human genome and encodes a protein that is essential for fetal survival will be the subject of intense research interest in the next few years.

Differentiation of trophoblast towards a more invasive phenotype early in pregnancy is thought to be stimulated by the comparatively low oxygen tension in the uterus. Exposure of first trimester human villous explants to low oxygen tension triggers trophoblast proliferation, fibronectin synthesis,  $\alpha_5$  integrin expression and gelatinase-A activity [10•]. These effects are, in part, mediated by transforming growth factor  $\beta_3$  (TGF $\beta_3$ ). They are inhibited following antisense expression of hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ), which instead triggers expression of markers of an invasive phenotype, such as  $\alpha_1$  integrin and gelatinase-B. Thus, the oxygen-dependent events of early trophoblast differentiation are, in part, mediated by TGF $\beta_3$  through HIF-1 transcription factors [10•].

### Cardiovascular development

Factors important in vasculogenesis and angiogenesis include vascular endothelial growth factor (VEGF), the related placental growth factor, their receptors (VEGFR-1 and VEGFR-2), angiopoietin and the angiopoietin receptors (Tie-1 and Tie-2) [11]. Null mutation of the aryl hydrocarbon nuclear translocator (ARNT), a transcriptional regulator that dimerizes with HIF-1 $\alpha$ , indicates that angiogenesis is differentially regulated in placenta, yolk sac and embryo [12•]. In *Arnt*<sup>-/-</sup> mice, abnormal development of the placental labyrinth was found to be associated with reduced VEGF binding and decreased expression of VEGFR-2. In contrast, vascular development was normal in the yolk sac and embryo, and seemed here to be dependent on VEGFR-1. HIF-2 $\alpha$  was also required for normal vascular development and appeared to be important for the vascular remodeling that follows vasculogenesis and angiogenesis [13•].

Hyaluronic acid has been assigned a role in cell migration and tissue remodeling. Mice null-mutant for hyaluronan synthase 2 had severe cardiovascular anomalies. The yolk sac endoderm and mesoderm failed to fuse and vitelline vessels were lacking [14].

Components of the TGF $\beta$  family and its signaling system are essential for left–right patterning. Their absence leads to cardiovascular anomalies that result in embryonic death [15]. Left–right patterning of abdominal organs is separately controlled. Abnormal displacement of the viscera to the opposite side of the body (situs inversus) occurs in the absence of growth/differentiation factor 1 [16•]. Although *Gdf1*<sup>-/-</sup> mutants can show aberrant left–right patterning of the heart and lungs, this is independent of whether or not positioning of the abdominal organs is affected [16•].

Finally, adrenomedullin — encoded by *Adm* and best known as a potent vasodilator — may play a role in cardiovascular development. *Adm*<sup>-/-</sup> mutants die at midgestation with extreme hydrops fetalis and cardiovascular abnormalities that include hypertrophied ventricular trabeculae and underdeveloped arterial walls [17]. Although there was a marked increase in amniotic fluid volume, *Adm* expression in fetal membranes was not examined. However, adrenomedullin has been demonstrated in human amniotic epithelium by immunohistochemistry [18].

### Maternal placental vasculature

In human and primate placentae, the maternal blood supply is from the spiral arteries of the uterine wall. These open directly into the intervillous space, where maternal blood comes in direct contact with the syncytiotrophoblast of the fetal villi (hemochorial placentation). Remodeling and widening of the spiral arteries is essential to ensure an adequate maternal blood supply to the uterus and includes replacement of the vessel endothelium with extravillous trophoblast. Tie-2 receptor is expressed in these endovascular trophoblasts and in maternal endothelium, suggesting a role for angiopoietin-2 in remodeling of the maternal vessels [19].

Vascular remodeling of uterine arteries without trophoblast invasion is an interesting feature of mouse pregnancy. It is known to be dependent on uterine natural killer cells (uNK cells), as pregnancy-induced changes in the arteries are not initiated in mice that fail to develop uNK cells and this effect can be reversed by uNK cell grafts [20]. Arterial remodeling is dependent on interferon- $\gamma$  (IFN $\gamma$ ) secretion from uNK cells and occurs normally following administration of recombinant IFN $\gamma$  to mice that lack uNK cells [21]. Concurrent with uterine vascular development, VEGF mRNA and protein increase in the decidua and the metrial gland, where the uNK cells reside, and it was shown by immunohistochemistry that VEGF was located to uNK cells [22].

As many as 10% of all genes are subject to parental imprinting, the monoallelic expression of genes depending on parental legacy. Several of these imprinted genes are critical to placental development. In the case of the *Igf2* gene, the phenotype of the offspring is determined by the paternal allele. In contrast, the phenotype of the IGF clearance receptor, *Igf2r*, is determined by the maternal allele [1]. Recently it was found that there is another set of

imprinted genes, on mouse chromosome 12, that is needed for normal trophoblast development, vascularization and invasion. Interestingly, these genes impact on fetal-maternal interactions. Thus, transformation of the wall of the maternal artery was not complete in pregnancies where a conceptus had been generated with paternal uniparental disomy for chromosome 12 [23\*\*].

Inadequate remodeling of the spiral arteries, due to failure of a second wave of trophoblast invasion, has been linked to preeclampsia. This disease is characterized by widespread endothelial dysfunction and is caused by a circulating factor or factors of placental origin. Incubation of myometrial arteries from normotensive pregnant women with plasma from women with preeclampsia caused a large reduction in endothelium-dependent regulation [24]. A similar effect was seen when the vessels were incubated with VEGF, but neither VEGF nor preeclamptic plasma affected vascular reactivity in the presence of an antibody to the VEGFR-1. This suggests that placentally derived VEGF and its receptor play a central role in the pathogenesis of preeclampsia [25].

## Important factors in fetal growth

### Insulin-like growth factors

IGF-I and IGF-II are mitogenic peptides that also promote differentiation, migration and aggregation and inhibit apoptosis. Their biological roles in fetal and placental development have been further delineated by *in vivo* and *in vitro* studies. As shown recently for the mouse [26] and non-human primate [27], these peptides are synthesized at specific times and locations during development to exert their regulatory function in an autocrine and/or paracrine manner.

Birth weight in humans has been correlated positively to IGF-I levels in umbilical cord blood [28]. Placental weight was shown recently to correlate with the ratio between IGF-II in cord blood and a soluble, circulating form of the IGF type 2 receptor (IGF2R) that inhibits IGF-II-mediated DNA synthesis [29]. Placental weight also correlated with levels of IGF-I and of IGF binding protein (IGFBP)-3 in cord blood [29].

IGF-II has recently been implicated in the fetal overgrowth that occurs in cattle following *in vitro* fertilization and embryo transfer. IGF-II mRNA levels were twofold greater in these fetuses than in controls when measured at day 70 of gestation [30].

The biological effects of IGFs are mediated by the type 1 IGF receptor (IGF-1R), the IGF-II/mannose-6-phosphate receptor and the insulin receptor. *Igf1r*<sup>-/-</sup> newborn mice are severely growth-restricted and die postnatally because of respiratory problems [31]. However, when the *Igf1r* gene was targeted using a construct that reduced IGF-binding sites by 41% in the homozygotes, prenatal and immediate postnatal growth was normal [32].

The bioavailability and biological actions of IGFs are regulated by a family of six high-affinity IGF binding

proteins (IGFBP-1 to -6) [33]. The *Igfbp* genes are considered not to be growth control genes [1]. Accordingly, *Igfbp2*<sup>-/-</sup> mice have normal birth weights [34] and even mice with combinatorial knockouts of IGFBPs fail to show a fetal phenotype [35]. However, the fetus responds to hypoxia, nutrient deprivation and stress by upregulating IGFBPs, thereby restricting fetal growth [36,37]. Subjecting *Igfbp*<sup>-/-</sup> mice to growth-restrictive conditions may result in different outcomes compared with wild-type mice. These may be useful models to study the role of IGFBPs in fetal growth.

### Leptin

Leptin is a hormone secreted by adipose tissue that regulates food intake and energy balance. The placenta is one of the major sources of leptin in the fetal circulation [38]. There is no clear evidence that leptin affects fetal growth but growth-restricted human fetuses do have reduced leptin expression in the placenta and lower leptin levels in cord blood [39]. A role for leptin in mouse development is suggested by the recent demonstration of leptin receptors in various fetal tissues of the mouse [40].

## Fetal programming

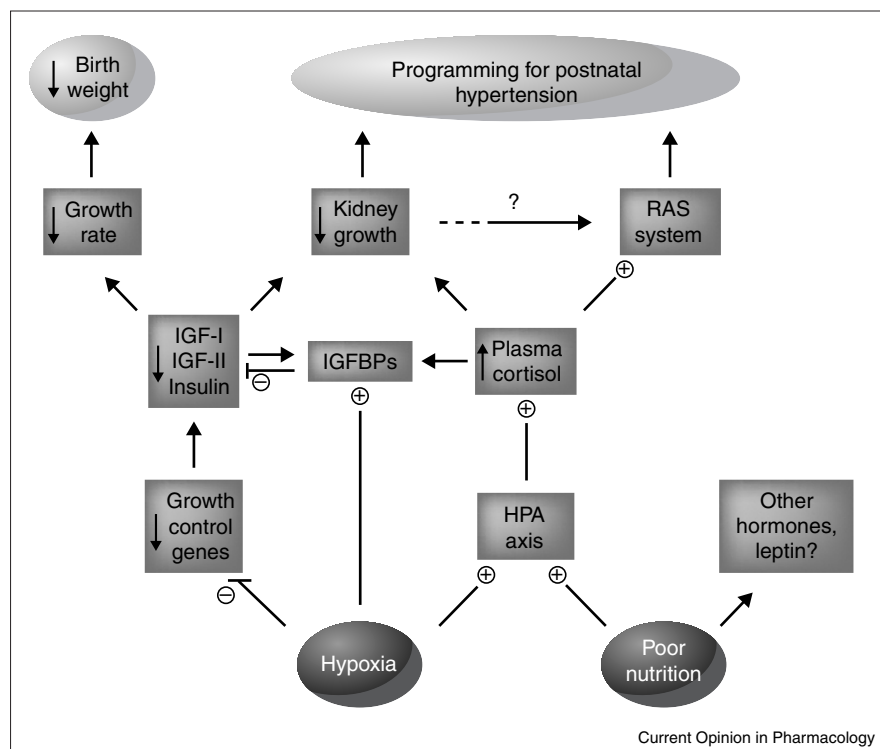
Poor nutrition or lack of oxygen during fetal development will impact on fetal growth rate, resulting in lower birth weight and increased perinatal morbidity. In addition, it is recognized that developmental alterations associated with growth restriction may have long-term consequences. Thus, low weight at birth has been linked to an increased risk for adult-onset diseases, including hypertension, ischemic heart disease and non-insulin-dependent diabetes mellitus [2]. Because of its implications for adult health and disease, 'fetal programming' is the focus of much current research in fetal physiology. Glucocorticoids have been ascribed a pivotal role in fetal programming. Thus, exposure of fetal sheep to dexamethasone for 2 days at day 27 of gestation (term is ~150 days) was sufficient to trigger hypertension that persisted until the adults were at least 5 years of age. These animals did not develop insulin resistance, suggesting different timing for the programming of fetal blood pressure and in sensitivity to insulin [41•]. Dietary restriction in rats also caused fetal growth restriction. The offspring had high blood pressure and increased plasma levels of insulin and leptin. In addition, they were hyperphagic and become obese. These effects were ascribed to impaired neuroendocrine regulation as a result of fetal undernutrition [42].

To fully understand the link between low birth weight and adult-onset diseases, account must be taken of the factors that control fetal growth and those that impact on organ systems critical to blood pressure control and glucose homeostasis. Glucocorticoids have been assigned a central role in fetal programming, but there must necessarily be interaction with the IGF system (Figure 1).

The kidney may play an important role in prenatal programming of hypertension. The number of glomeruli in



Figure 1



Fetal programming is thought to explain the correlation between low birth weight and adult hypertension. The IGF system and the hypothalamic–pituitary–adrenal (HPA) axis are likely to be equally important in this context. Growth control genes include those coding for insulin, IGF-I and IGF-II. Fetal responses to hypoxia include downregulation of these genes and increased expression of inhibitory IGFBPs. Hypoxia also activates the HPA axis, raising plasma levels of adrenocorticotrophic hormone (ACTH) and cortisol. This is a further mechanism for regulating IGFBP expression. Kidney growth is affected by reduced IGF action, and possibly by increased cortisol levels, resulting in a reduced number of glomeruli. There are also alterations in the renin–angiotensin system (RAS), probably downstream to activation of the HPA axis. It is not known if renin secretion from the juxtaglomerular apparatus decreases in parallel with the decrease in glomerular number; however, the alterations in RAS function and compensatory responses for reduced kidney function probably account for the predisposition to adult hypertension. Poor fetal nutrition and other stress factors act through similar mechanisms.

the kidney was found to be reduced by 28–29% in rats that had low birth weight due to dietary restriction, but had achieved full catch-up growth during the first weeks of life [43<sup>\*</sup>]. A progressive deterioration in renal function was seen in hypertensive rats subjected to growth restriction in fetal life, suggesting that hemodynamic adaptations to maintain renal function following the reduced nephrogenesis caused a more rapid progression to renal failure [44]. Rodent experiments have further suggested involvement of the renin–angiotensin system in fetal programming for hypertension, possibly acting downstream of glucocorticoids. In support of this, the hypertension that develops in rats following dietary restriction in pregnancy can be prevented by treatment with losartan, an angiotensin AT<sub>1</sub> receptor antagonist, between 2 and 4 weeks postnatally. The implication of this experiment is that fetal influences may alter postnatal vascular responsiveness [45].

Moderate maternal exercise started early in pregnancy, during the phase of rapid placental growth, may be a strategy to improve fetal well-being. In a recent controlled study, the offspring of exercising women were found to be significantly heavier and longer at birth [46<sup>\*</sup>]. It remains to be determined whether a similar exercise regimen would prove beneficial in women who are at sociodemographic risk of having a low birth weight infant.

## Conclusions and future directions

### DNA microarray technology

Our understanding of how fetal and placental growth is controlled is likely to be enhanced by DNA microarray

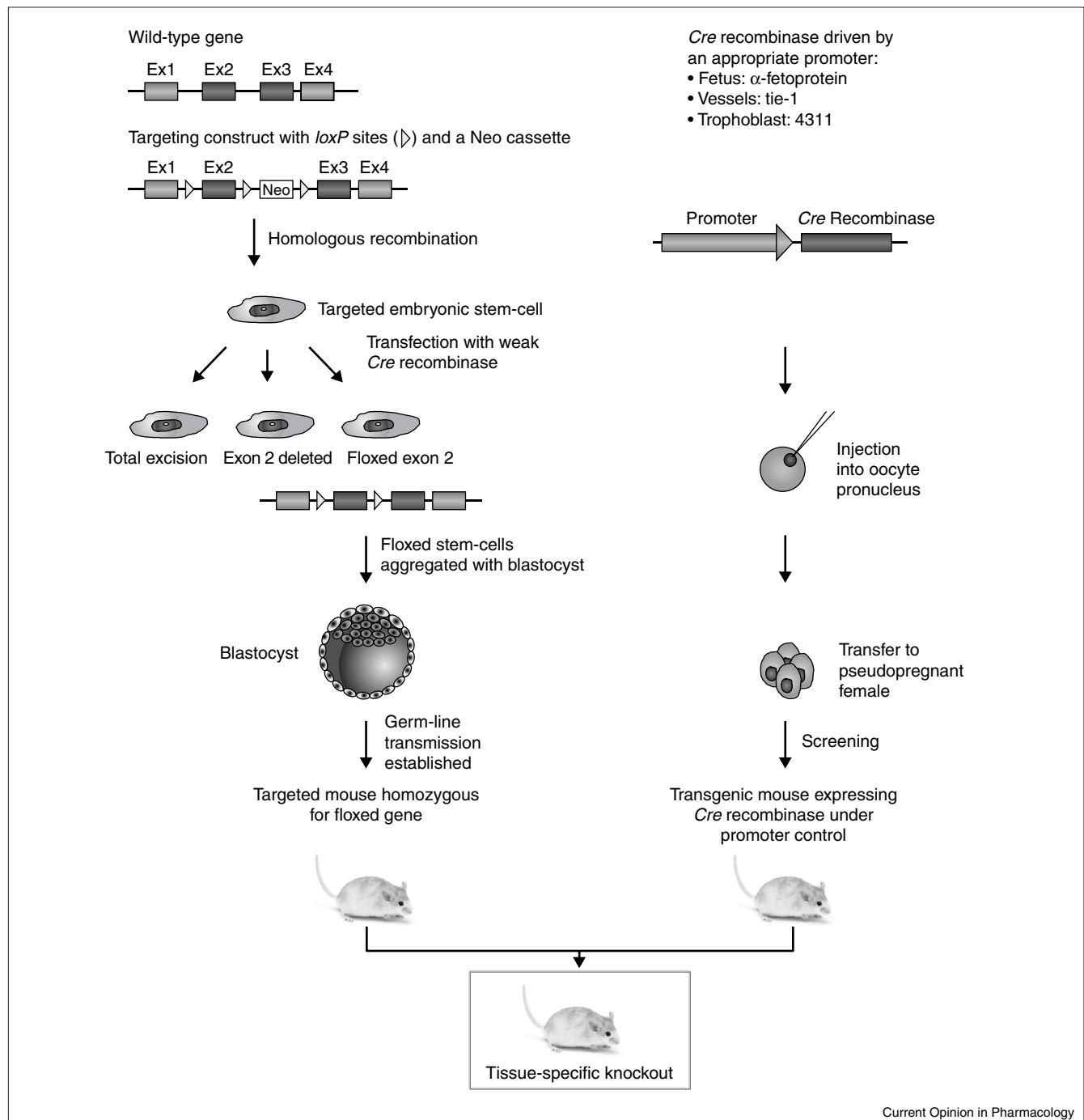
technology. There is now a cDNA microarray that contains more than 15 000 genes expressed during development in mice. An initial application to the midgestation (E12.5) conceptus identified 289 genes that were more highly expressed in the placenta than in the embryo, only 30 of which had previously been reported [47<sup>\*\*</sup>]. Potential applications of microarray technology include detecting genes that change expression during embryonic or extraembryonic development and identifying downstream target genes in transgenic and knockout mice [47<sup>\*\*</sup>].

### Tissue-specific gene targeting

Tissue-specific gene activation and inactivation is now possible using the *Cre*-recombinase-*loxP* (*Cre-loxP*) system. *Cre* recombinase is an enzyme of bacteriophage origin that catalyses recombination between two of its recognition sites, *loxP*. Insertion of *loxP* sites into a gene can usually — although not inevitably — be achieved without affecting gene expression. Excision of the region flanked by the *loxP* sites will occur only upon expression of *Cre* recombinase, which can be driven by a tissue-specific promoter. This will activate or, more usually, inactivate the gene (conditional knockout) [48]. The simplest strategy to achieve this is to cross a *loxP* provider mouse line and a *Cre*-expressing transgenic line (Figure 2).

Application of this methodology to fetal and placental development has hitherto been limited, because *Cre* transgenic mice that have been developed so far use promoters that are activated postnatally or only late in fetal development. A

Figure 2



Strategy for conditional knockout of a target gene in fetal or placental tissues. The left-hand panel shows the process for developing a targeted mouse homozygous for a floxed gene. The wild-type gene of interest is selected and a targeting construct made, in which an essential exon (Ex2) is flanked with *loxP* sites by gene replacement. A neomycin-resistance cassette (Neo) equipped with a third *loxP* site is co-introduced. The construct is then inserted into embryonic stem cells by electroporation. Stem cells with homologous recombination are selected and grown. These cells are transfected with a weak *Cre* recombinase, with three possible results: total excision of exon 2 and the Neo cassette; excision of exon 2; and excision of the Neo cassette. The latter cells (i.e. with floxed exon 2) are selected and aggregated with a blastocyst, which is transferred to the uterus of a

pseudopregnant mouse. Finally, a breeding program to establish germ-line transmission yields a targeted line homozygous for the floxed gene. The right-hand panel shows the production of a transgenic mouse expressing *Cre* recombinase under the control of a promoter. The *Cre* recombinase gene is linked to a promoter – for example,  $\alpha$ -fetoprotein promoter for fetal liver, the *tie-1* promoter for fetal vessel endothelium or the 4311 promoter for spongiotrophoblast. Following microinjection of the construct into an oocyte, the 2–4 cell embryo is transferred to the uterus of a pseudopregnant mouse. A breeding and screening program yields a transgenic line expressing *Cre* recombinase under the chosen promoter. Crossing the floxed targeted mouse with the transgenic mouse expressing *Cre* recombinase with the desired promoter results in the tissue-specific deletion of exon 2.

*tie-1-Cre* transgenic strain — where the enzyme is expressed in endothelial cells of the embryo, yolk sac and placenta from E10 until term — will allow direct deletion of floxed genes involved in vasculogenesis and angiogenesis [49]. Transgenic mice with embryonic-cell or placental-cell specific *Cre* recombinases are currently under development (Figure 2).

### Gene-dosage effects

The *Cre-loxP* system can also be used to explore gene-dosage effects. A variable reduction in IGF-I levels has been achieved by crossing mice with a *loxP*-flanked *Igf1* locus and mice expressing *Cre* recombinase under an adenovirus promoter that is expressed early in embryonic development [50]. In mice with the most severe recombination (*Igf1* gene dosage 10–40% of normal), birth weight was reduced by 31%, as in the classical *Igf1* knockout experiment [31]. However, mice with *Cre*-induced partial recombination (gene dosage 50–90% of normal) had a birth weight reduction of only 12% and a phenotype that allowed postnatal survival [50].

### In utero gene transfer

Developmental disorders that can be diagnosed early in gestation may become treatable *in utero* by gene therapy. Gene transfer to multiple organs and transgene expression has been achieved after vector administration via the peritoneal cavity to rhesus monkey fetuses [51]. Moreover, treatment of fetal monkeys with an adenovirus expressing the cystic fibrosis transmembrane conductance regulator (*Cfr*) gene has been shown to result in accelerated lung differentiation [52\*]. Finally, as gene transfer to mice at E15 results in prolonged transgene expression [53], this technique could be exploited as an experimental tool in future studies of fetal growth and development.

### Update

A recent study advances our understanding of how trophoblast differentiation is directed in mouse placenta [67\*]. Expression of nodal (a member of the TGF- $\beta$  family) is restricted to the spongiotrophoblasts and may direct trophoblast cell fate towards development of the labyrinth. *Nodal*<sup>−/−</sup> placentae have an excessive number of giant cells with total loss of the spongiotrophoblast and labyrinth layers. A hypomorphic nodal allele has now been generated by insertion of *loxP* sites. In placentae heterozygous for the null allele and the hypomorphic allele, all cell layers were present. However, the giant-cell layer and the spongiotrophoblast layer were increased in thickness at the expense of the labyrinth. Thus, *Nodal* expression by the spongiotrophoblasts may direct labyrinth development from one side, while signals at the interface of chorion and allantois, such as *Gcm1* [5\*], direct it from the other.

The critical role of VEGF in fetal and placental development has been further highlighted by null mutation of the *Lkb1* gene, which encodes a serine/threonine kinase [68]. *Lkb1*<sup>−/−</sup> mice exhibited multiple embryonic abnormalities, poor yolk-sac vascularization and a complete absence of fetal

vessels in the placenta. Death ensued at E8.5–11. This phenotype was associated with deregulation of VEGF in the embryonic and extraembryonic compartments.

The programming effects of prenatal glucocorticoid exposure may not depend on a reduction in fetal growth. In a carefully controlled study, glucose homeostasis in lambs was affected by betamethasone administration to either mother or fetus during late gestation. Yet fetal growth restriction and altered blood pressure control occurred only when glucocorticoids had been given to the mother [69\*]. These findings add further support to the view [41\*] that programming of arterial blood pressure and glucose tolerance are not the result of a common mechanism.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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- of outstanding interest

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